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Degradation of lignocellulosic content of rice straw using aerobic cellulolytic bacteria isolated from forest soil of Bangladesh

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The present research work was conducted to enumerate and isolate aerobic cellulolytic bacteria from forest soil using carboxymethyl cellulose (CMC) as substrate. The identified isolate was further tested as potent candidate to improve the nutritional value of rice straw. The forest soil was loaded with substantial amount (2.45×10^7) of cellulolytic bacteria. A total of 10 cellulose degrading bacteria (CDB) were isolated, identified and monitored for their cellulolytic activity. The isolate having the highest cellulolytic index (2.5) was identified as *Bacillus subtilis* targeting the 16S rRNA gene which was labeled as *B. subtilis* strain CDB7 and investigated for nutritional improvement of rice straw. Solid state fermentation of each group was carried out at 37°C for a period of 0, 3 and 6 days in 0 (no bacterial inoculum), 1, 5 and 10% inoculum group. There were a tendency of reduction in Acid Detergent Fiber (ADF), Neutral Detergent Fiber (NDF) and lignin at concentrations of 1, 5 and 10% inoculums at every interval of fermentation. Highest reduction of ADF (4.8%), NDF (10.78%) and lignin (37.6%) were observed after 6 days of fermentation at 10, 10 and 5% inoculum group, respectively. The crude protein content was increased (5.3 ± 0.4 to 6.4± 0.3) in 10% inoculum group. Taken together, the identified isolate could be a potent candidate to degrade lignocellulosic content through breaking of lignin-cellulose bondage and to improve the nutritional value of rice straw.

Key words: Rice straw, *Bacillus subtilis* strain CDB7, solid state fermentation, acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin.

INTRODUCTION

The global demand for livestock products is expected to double by 2050 (Conforti, 2011). However, the acute scarcity of feeds and fodder has been considered as one of the biggest problems in the development of livestock

production. Because more than 70% expenditures are incurred on feed while raising livestock, a huge gap in demand and supply of feeds and fodders in many developing countries including Bangladesh has already been reported (Huque and Sarker, 2014). Ruminant nutrition is mostly dependent on naturally grown grasses, various crop residues like straws, stovers, stalks and cereal by-products. Rice straw is considered as the prime crop residue roughage feed which farmers usually store and use in tropical areas especially during the long dry season. However, rice straw is often termed as very poor quality roughage feed due to low protein content (2 to 5%), fiber and lignin contents (Neutral Detergent Fiber (NDF) > 50%) and also having low digestibility (<60%). The microbial treatments of rice straw can improve the accessibility and is considered as a cheap and sustainable approach. Thus improving their digestibility and feeding value have been attracting the extensive interests among researchers (Zhang and Lynd, 2004); although this process has a long history. The reason behind the low degradability, rice straw consists of cellulose, hemicelluloses and lignin that are strongly intermeshed and chemically bonded in its structure. So feeding rice straw only provides inadequate nutrients for optimum production requirements for livestock (Wanapat et al., 2013). However, the use of crop residue as animal feed is limited due to its structure, low nutritive value, and high structural carbohydrate content (Chanjula et al., 2017).

The breakdown of cellulose is performed by the enzyme called cellulase which is generally secreted by anaerobic cellulolytic bacteria found in the rumen. They also produce some lignocellulytic enzymes which make more easily available nutrients to animal. These enzymes also were reported to be produced by fungi, bacteria, and protozoans existing in the environmental samples (Immanuel et al., 2006). This enzyme is produced by several microorganisms, commonly by many cellulolytic bacteria. Cellulolytic enzymes are synthesized by a number of microorganisms. Abdel-Rahman et al. (2016) showed an efficient composting process of rice straw when inoculating with mixed culture of *Bacillus* species that reduced the composting time by 40 to 43%.

For aerobic and anaerobic mesophilic bacteria, certain protozoa are able to utilize lignocellulose in different fibrous feed (Alexander, 1978) and they play an important role in conversion of lignocelluloses in straw into readily available nutrients for the animal.

The major obstruction in biological conversion of lignocelluloses is the physical protection of cellulose by lignin against cellulolytic enzymes. As lignocellulytic bacteria have been isolated and characterized from variety of sources such as soil, organic matters, decayed plant materials, feces of ruminants and composts, therefore, soil might be the good source of aerobic cellulolytic bacteria. Cellulolytic properties of some bacterial genera such as *Cellulomonas*, *Pseudomonas*, *Bacillus* and *Micrococcus* species were reported (Abou-Taleb et al., 2009). Recently, probiotics are randomly used to increase the nutritional value of fiber constituents of roughage. So, cellulolytic bacteria can play an active role to degrade the fibrous part (Acid Detergent Fiber (ADF), NDF and lignin) of rice straw by producing the enzyme. This will convert fiber into readily available nutrients.

The aim of this study was to improve the nutritional value of rice straw to make more available nutrients for ruminants by using cellulase producing aerobic cellulytic bacteria from forest soil.

MATERIALS AND METHODS

Soil sample collection and preparation

Soil samples were collected from the Bhawal National Forest, Gazipur, Bangladesh. For each sample, soil was first dug out with clean shovel up to 10 cm depth using disposable and sterile spatula. Soil was then mixed thoroughly and foreign materials like tree roots, leaves were removed. Approximately 50 g was transferred to a sterile zip lock bag. All the soil samples after collection were properly sealed, labeled and sent to the laboratory where they were kept at 4°C. At first the soil sample was ground to fine particles and 10 g of the sample was added in 90 mL of distilled water and was shaken thoroughly. After the proper mixing, the suspension was filtered to remove the unwanted portion of the soil. The filtrated suspension was used for the isolation of bacteria.

Enumeration and isolation of cellulolytic bacteria

The carboxymethylcellulose (CMC) agar medium containing 1.0% peptone, 1.0% CMC, 0.2% K_2HPO_4, 1% agar, 0.03% $MgSO_4.7H_2O$, 0.25% $(NH_4)_2SO_4$ and 0.2% gelatin adjusted to pH 7.0 was used for enumeration as well as isolation of aerobic cellulolytic bacteria. Serially diluted soil suspension was spread onto CMC agar plate and incubated at 37°C for 48 h. The total cellulolytic bacterial load was expressed as colony forming unit per gram (cfu/g). For isolation, from each highest dilution, plate colonies having different shapes were selected and purified by repeated streaking onto the same agar media. The purified isolates were Gram stained and then preserved at 4°C in agar slant media for further study.

Congo red counter staining

After grown overnight in nutrient broth, each pure isolate was

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Table 1. Total viable cellulose-degrading aerobic bacterial load.

Type of soil	Viable bacterial load (log cfu/g)
Forest soil	7.4[a]
Farming soil	5.1[b]

Means with different superscripts within a column are significantly different ($p < 0.05$).

spread onto CMC agar plates followed by incubation at 37°C for 48 h. The CMC agar plates were then flooded with 1% Congo red and allowed to stand for 15 min at room temperature. The plates were counterstained with 1 M NaCl solution. Clear zones appeared around grown colonies indicating cellulose hydrolysis.

Measurement of hydrolytic capacity

The hydrolytic capacity of each isolate was determined by measuring the diameter of the colony and surrounded clear zone. The hydrolytic capacity was expressed as cellulytic index calculated from the differences of diameter of hydrolytic zone and bacterial colony divided by diameter of bacterial colony (Ferbiyanto et al., 2015). The isolate having the highest hydrolytic capacity was selected for molecular identification and degradation ability of rice straw under solid-state-fermentation.

Molecular identification

The isolate having the highest hydrolytic capacity was used for molecular identification. The isolate was cultured in nutrient broth at 37°C for 48 h. After incubation, the genomic DNA of the isolate was extracted using a commercial DNA Purification Kit (Thermo Fisher Scientific, USA) following the manufacturer's protocol. The PCR reaction mixture was prepared as a volume of 100 µL containing 6 µL of 25 mM $MgCl_2$ (Thermo Fisher Scientific, USA), 10 µL of 10× Dream *Taq* buffer (Thermo Fisher Scientific, USA), 2 µL of 10 mM dNTP mix (Thermo Fisher Scientific, USA), 5 µL each of forward primer (27F: 5'-AGAGTTTGATCATGGCTCAG-3') and reverse primer (1492R: 5'-AGGAGGTGATCCAACCGCA-3') (Thermo Fisher Scientific, USA), 5 µL of template DNA, 1 µL of *Taq*DNA polymerase (Thermo Fisher Scientific, USA) and 66 µL of nuclease-free water were used for the amplification of 16s rRNA universal sequence (Frank et al., 2008). The PCR amplification of 16S rRNA gene was performed in a PCR Thermocycler (Applied Biosystems, Thermo Fisher Scientific, USA). The thermal profile for PCR was set as follows: an initial denaturation step at 94°C for 5 min; 35 cycles of a denaturation step at 94°C for 1 min, an annealing at 57°C for 40 s and an extension at 72°C for 1 min and a final extension step at 72°C for 10 min. The amplified PCR product was purified by using a commercial PCR Purification Kit (Thermo Fisher Scientific, USA) following the manufacturer's protocol. Raw sequence reads were analyzed by using MEGA 7.0. Sequence homologies of the bacterial isolates with closest similar isolates were determined by using web-based BLAST (Basic Local Alignment Search Tool) program of the NCBI website (ncbi.nlm.nih.gov/BLAST). After analysis, the FASTA sequences are submitted to the NCBI database for GenBank accession number. Phylogenetic trees were prepared by neighbor joining method in MEGA7.

Soild-state fermentation of rice straw

The solid-state-fermentation of rice straw was carried out using the isolate having highest hydrolytic capacity. The fermentation was arranged according to a factorial arrangement in a complete randomized design (CRD). A total of four treatments comprising (1) untreated rice straw, (2) rice straw treated with 1% inoculum, (3) rice straw treated with 5% inoculum, and (4) rice straw treated with 10% inoculum were performed. The rice straw collected from the local market was ground into 2 to 3 mm length using a mechanical grinder which was then sterilized by autoclaving at 121°C for 15 min. One hundred fifty grams of sterilized rice straw was taken in a Ziplock bag and mixed with sterilized distilled water at a ratio of 3:1. The isolate having the highest cellulolytic index was grown overnight in the CMC broth at 37°C for 48 h. Then, the CMC broth without bacteria added to the control group and CMC broth having 1.2×10^{10} cfu/ml bacteria was added at 1, 5 and 10% in the treatment group. Then, all the bags were placed in an incubator at 37°C and kept for a period of 6 days.

Estimation of changes in rice straw composition during fermentation

The changes of composition in rice straw were estimated at every 3 days interval of solid-state fermentation. The DM, CP, ash, ADF, NDF and lignin of rice straw in both control and treated groups were measured according to AOAC (2000).

Statistical analysis

Data were recorded, checked for completeness and consistency and subjected to analysis using Excel and R (4.0.2 version) software packages. One-way ANOVA was used for multiple mean comparisons. $P< 0.05$ was considered significant during the analysis.

RESULTS AND DISCUSSION

Viable cellulolytic bacterial load in soil samples

The total aerobic cellulolytic bacterial load in pooled forest soil samples were 7.4 log cfu/gm. The bacterial load was also compared with the farming soil sample (Table 1) which showed a significant difference among the samples suggesting the forest soil as a predominant source of cellulolytic bacteria.

The number of colonies in forest soil was higher than of the load of farming soil. Hatami et al. (2008) found the number of colonies forming unit was 138 in forest soil and in farming soil it was 126 which indicates forest soil contain more load of cellulytic bacteria. The organic matter in soil is utilized as energy and carbon resources by cellulolytic bacteria. Higher number of cellulolytic bacteria in forest soil can be attributed to higher organic carbon content in forest soils. The number of cellulolytic bacteria in forest soil samples is more than farm soils due to the type of organic matter in forest soils. This also may

Table 2. Characteristics of cellulose-degrading aerobic bacteria from the forest soil.

Name of isolate	Colony characteristics		Cellulytic index
	Morphology	Gram staining	
CDB1	Thin, watery, small round colony	Negative	ND
CDB2	Watery, medium round colony	Negative	ND
CDB3	Thick, round, light cream color colony	Negative	0.26
CDB4	Thick, round, light cream color colony	Negative	0.62
CDB5	Very small round shaped, pinpoint colony	Negative	1.04
CDB6	round shaped, pinpoint colony	positive	ND
CDB7	Large, round but outside branching, thick, cream color colony	positive	2.50
CDB8	Thick, round, light cream color colony	Negative	ND
CDB9	Thick, round, light cream color colony	Negative	ND
CDB10	Thin, round, light cream color colony	positive	ND

ND: Not detected.

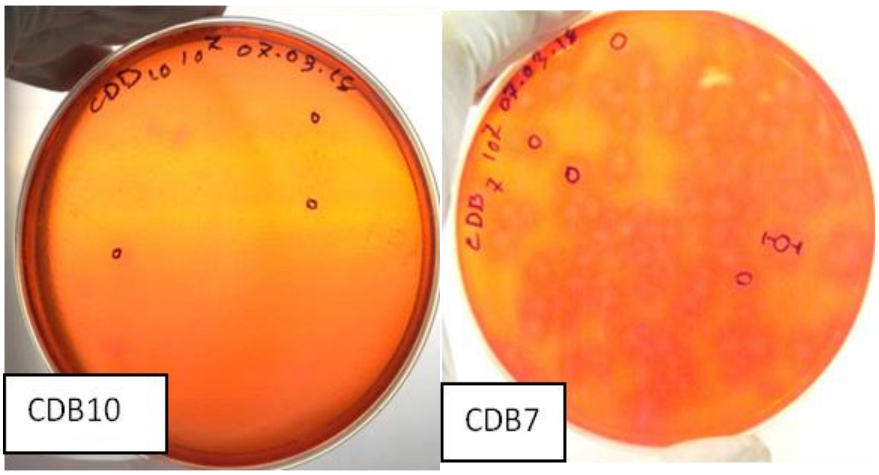

Figure 1. Representative cellulolytic index (z-c)/c (mm) of different CDB isolates.

be due to the fact that micronutrients discharged into the forest soil through plant wastes reduce the porosity of the soil.

Morphological characteristics and hydrolytic capacity of the isolate

After spreading forest soil suspension on CMC agar plates, a total of 10 colonies were isolated randomly. The isolates were labeled as CDB1, 2, 3….CDB10. As shown in Table 2, the isolates varied in shape and color (round, elongated, watery, and cream). Of the 10 isolates, 3 were identified as Gram positive and the remaining 7 were Gram negative. Out of the 10 isolates, only 4 showed hydrolytic capacities and produced a clear hydrolytic zone around the colonies (Figure 1). Conversely, the isolates having less/no hydrolytic capacity showed absence of such zone (Figure 1). The highest cellulolytic index was observed by the isolate CDB 7 indicating the ability to produce cellulase enzyme. Consequently, the isolate was selected to test as a candidate for improving the nutritional value of rice straw and also to identify at molecular level.

In the cellulolytic Index study, the highest value was 2.5 found in CDB7 and the lowest value was 0.2 in CDB3. This finding was similar to Ferbiyanto et al. (2015). Ferbiyanto et al. (2015) measure cellulolytic activity of bacterial isolates based on a clear zone of degraded CMC area around the colony. Cellulolytic activity test showed that the isolate has the largest cellulolytic index of 2.5 and the isolate has the smallest cellulolytic index of 0.75. Cellulytic index is an indicator of producing cellulase enzyme and based on these criteria, CDB7 was

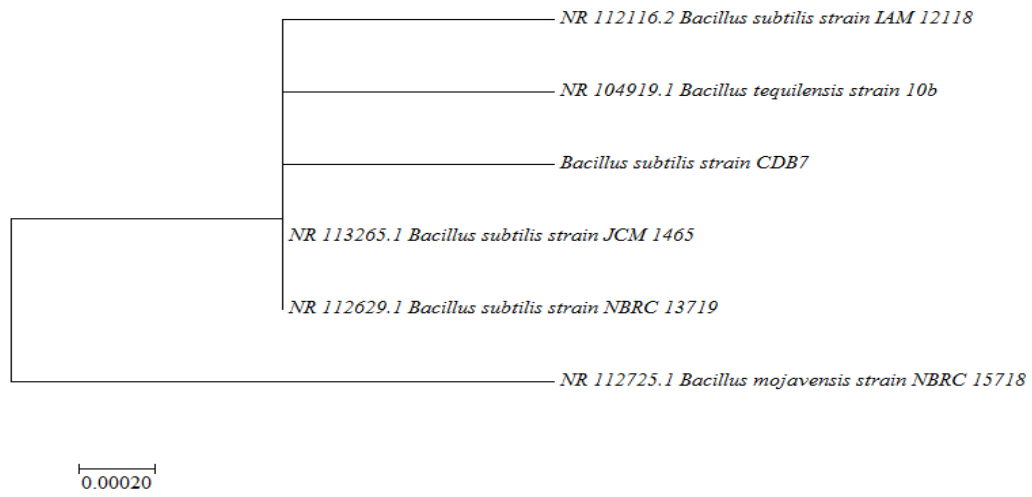

Figure 2. Phylogenetic tree of *Bacillus subtilis* strain CDB7.

selected to be used in the solid state fermentation. CDB7 showed hydrolysis capacity of 2.5 (Table 2) in the Congo red assay having similarities with Khianngam et al. (2009) who isolated cellulolytic bacteria of *Bacillus*, *Lysinibacillus* and *Paenibacillus* genus from the sample of oil palm meal, which showed cellulolytic index in the range from 1.56 to 4.14 in the Congo red assay. Maki et al. (2011) showed that cellulolytic bacteria were isolated from soil samples from natural reserves producing zones of hydrolysis in Congo red method and found *Paenibacillus terrae* ME27 as the highest cellulase producer. The appearance of the clear zone around the colony when the Congo red solution was added (Wood and Bhat, 1988) was strong evidence that the bacteria produced cellulase in order to degrade cellulose.

Hydrolytic capacity of the isolates

Four isolates (CDB3, CDB4, CDB5 and CDB7) out of ten showed hydrolyzing capacity on agar plates containing CMC as core carbon source, after Congo-red staining. The hydrolyzing zone diameter and colony diameter were listed as shown in Table 2.

In Figure 1, it is clear that cellulolytic bacteria produced cellulase enzymes and hydrolyzed the agar media around its colony. The bacterial colony produced a hydrolytic zone and showed differential cellulolytic index. In the case of *Bacillus subtilis* strain CDB7 colony's highest clear zone was found.

Molecular characterization

The 16S rRNA gene sequencing data of the isolate CDB7 exhibited 99.79% homology with *B. subtilis*. In the phylogenetic tree analysis, *B. subtilis* strain CDB7 formed clusters with its corresponding species (Figure 2). The accession number of *B. subtilis* strain CDB7 is MW159692.

Rice straw fermentation

According to the highest hydrolytic capacity and the result of biochemical test CDB7 was selected for the further fermentation study. These isolates were selected for the solid state fermentation in rice straw. At first the pure culture of CDB7 was grown in the CMC broth for 48 h and then applied to rice straw to be fermented.

The physical appearance (color and smell) of rice straw during solid state fermentation with the target isolate (CDB7) for a period of 6 days were observed. The color of straw changed from brownish yellow to yellowish green (Figure 3). The smell was slightly acidic in the treated group compared to untreated. These indicate bacteria might have some action on rice straw. However, no fungal appearance was observed in any group during the entire fermentation period.

Improvement of nutritional value of treated rice straw

During the solid state fermentation for a period of 6 days the proximate composition of both rice straw samples treated with or without the target isolates were measured at every 3-days interval. The parameters of the proximate component included dry matter (DM), ash and crude protein (CP) shown in Table 3.

Among the proximate component, the CP content was

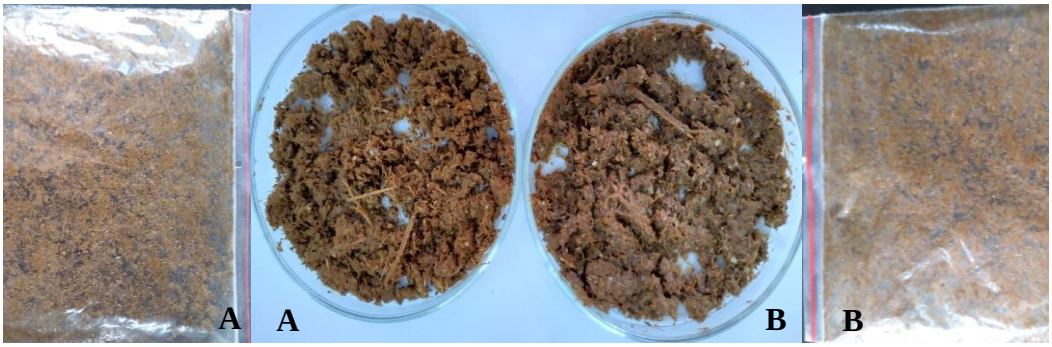

Figure 3. The change of color due to solid state fermentation of rice straw with *Bacillus subtilis* strain CDB7; A: Control, B: Treated.

Table 3. Proximate composition of the samples during solid state fermentation.

Inoculum level (%)	Fermentation period (days)	DM (%)	CP (% of DM)	Ash (% of DM)
0 (control)	0	23.4±0.4^{a}	5.4±0.6^{a}	16.1±1.2^{a}
	3	23.5±0.6^{a}	5.5±0.6^{a}	15.8±0.7^{a}
	6	23.5±1.16^{a}	5.4±0.5^{a}	15.4±1^{a}
1	0	21.51±0.8^{c}	5.5±0.4^{a}	16.8±1.2^{a}
	3	24.3±0.7^{a}	5.5±0.4^{ab}	14.6±0.7^{b}
	6	22.9±0.7^{b}	6.2±0.6^{b}	13.7±0.7^{c}
5	0	23.6±1.1^{a}	5.6±0.5^{a}	15.8±1.1^{a}
	3	22.1±1.2^{a}	6.2±0.8^{b}	14.2±0.7^{b}
	6	22.0±1.3^{a}	6.4±0.7b	13.1±0.5^{c}
10	0	22.9±1.1^{a}	5.3±0.4^{a}	16.5±0.9^{a}
	3	24.2±1.25^{a}	6.3±0.4^{b}	14.8±0.5^{b}
	6	23.7±2.17^{a}	6.4±0.3^{b}	15.5±0.2^{b}

*Values are expressed as mean ± SD (n = 3). Means with different superscripts within a column are significantly different ($p < 0.05$).

increased significantly with the increment of inoculum level as well as fermentation period as compared to control group. The highest CP% was observed after 6 days of fermentation in 10% inoculum groups (5.36±0.4 to 6.4±0.3). At the beginning of fermentation, the CP content was almost similar to all groups (5.3 to 5.6). The CP content was increased sharply after 3 days of fermentation and then the trend of increment was appeared to be static. These findings showed the similarities with the Sembiring et al. (2002), showed that the level of crude protein content of rice straw fermented with probiotic was 5.63 and it was in the range of other reports that was 5.63 to 11.25%.

The highest reduction (18.5%) of ash content was found in the 1% level of fermentation. The results are in agreement with Syamsu et al. (2013). Syamsu et al. (2013) showed that the ash value decreased from 22.34 to 20.19 (% of DM) when rice straw fermented with a starter of microbes. The present findings are also supported by Sariubang et al. (2002) who found the ash value decreased range 22.54-33.50% to 21.89-31.02% after treating with the probiotics. Akter et al. (2013) found that rice straw contained 15.02% ash which changed very little to 15.06, 15.09, 15.12 and 15.15% by the treatment with different concentration of urea and midden soil (3.0% urea + 2.0% midden soil, 3.0% urea + 3.0% midden soil, 3.0% urea + 4.0% midden soil and 3.0% urea + 5.0% midden soil, respectively).

ADF

ADF indicates the least digestible plant components which include cellulose and lignin and insoluble ash

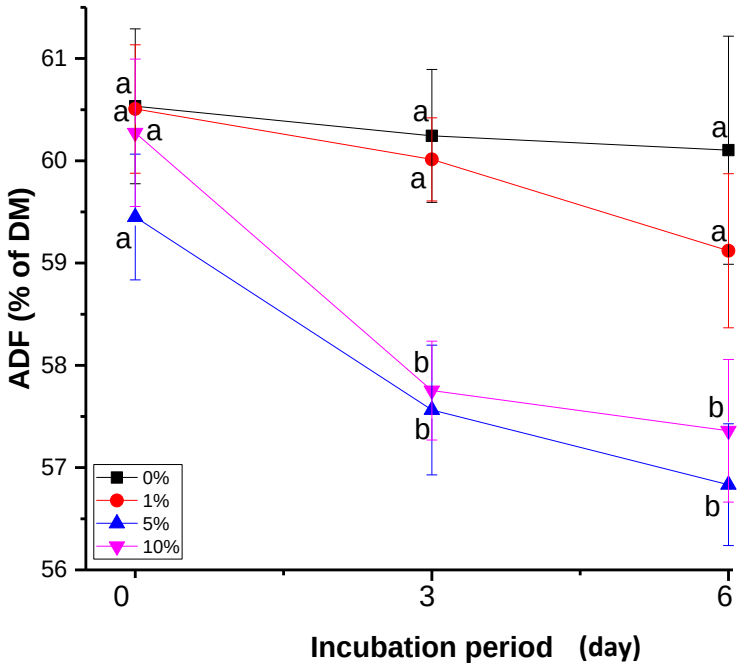

Figure 4. Degradation of ADF fraction of fiber in rice straw by *Bacillus subtilis* strain CDB7 isolates.

(mainly silica). The ADF values are inversely related to digestibility of a specific forage. Therefore, forages with low ADF concentrations usually contain higher amount of energy. The ADF value in the control and treated group of rice straw for 0, 3 and 6 days of fermentation is as shown in Figure 3. The ADF content was decreased from 60.5±0.6 to 59.1±0.8, 59.5±0.6 to 56.8±0.6 and 60.3±1.1 to 57.4±0.7 in 1, 5 and 10% inoculum level group, respectively. In control group, the ADF content was almost unchanged throughout the fermentation period. The highest reduction of ADF (4.8%) was observed in 10% of inoculum level group after 6 days of fermentation.

Figure 4 shows that ADF value was affected by treatment with different concentrations of inoculums at 0, 3 and 6 days showing similarities with Syamsu et al. (2013), who found that the ADF value decreased from 57.76 to 47.64 when treated with microbial starter.

NDF

NDF indicates structural components of the plant specially the cell wall which contains hemicellulose, cellulose, lignin, and insoluble ash. The level of NDF in the animal ration influences the animal's intake of dry matter. It is an important parameter to measure the relative feed value. As shown in Figure 4, the NDF value of rice straw decreased greatly from 90.7±1.1 to 87.7±1.4, 91.1±0.7 to 81.6±1.7 and 90±0.9 to 80.3±1.4 in 1, 5, and 10% of inoculums group, respectively. The highest percentage (10.78%) of NDF reduction was observed after 6 days of fermentation in 10% inoculums group (Figure 5).

These results of NDF are in agreement with the results of Bansi et al. (2012) and Sariubang et al. (2002). Bansi et al. (2012) showed that the decreased crude fiber level of rice straw fermented with commercial probiotic (P1) was decreased 6.07% than control. It was lower than reported by Sairubang et al. (2002) who reported that crude fiber content in rice straw fermented using probiotics decreased by 25.73 and 14.79%. Sariubang et al. (2002) found that the NDF value decreased from 79.78 to 77.0 when treated with probiotics.

The NDF was also decreased about 2% when compared with untreated sugarcane bagasse after treatment with a combination of cellulase, TH14 and molasses (Yoo et al., 2020). Though enzymes were often added with a variety of bacterial inoculations in their experiment, the decreased cellulose and lignin level of rice straw fermented interpret that probiotic microbes are able to penetrate the fibrolytic structure of rice straw and detach the binding of lignified carbohydrate and in some extent, degrade cellulose and hemicellulose. Selim et al. (2017) showed that the NDF content was reduced by probiotics at days 2 to 4. It was also reduced by urea, *Trichoderma* and *Aspergillus* treatments at all-time points.

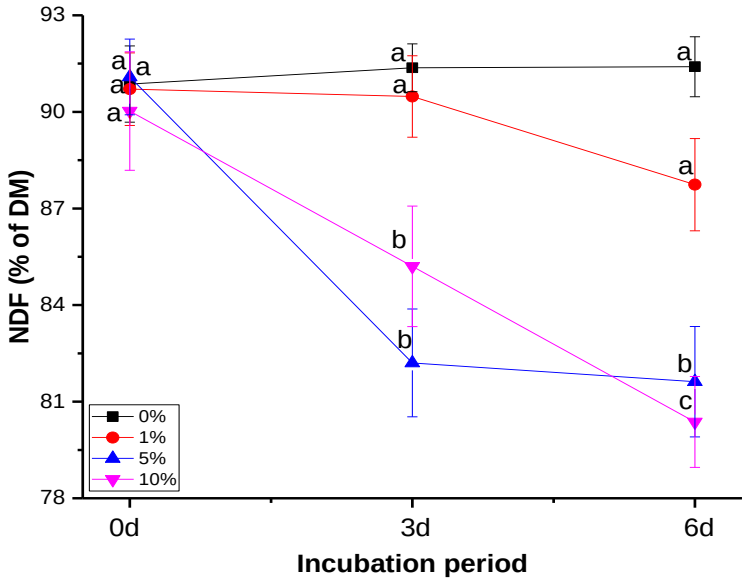

Figure 5. Degradation of NDF fraction of fiber in rice straw by *Bacillus subtilis strain CDB7* isolates.

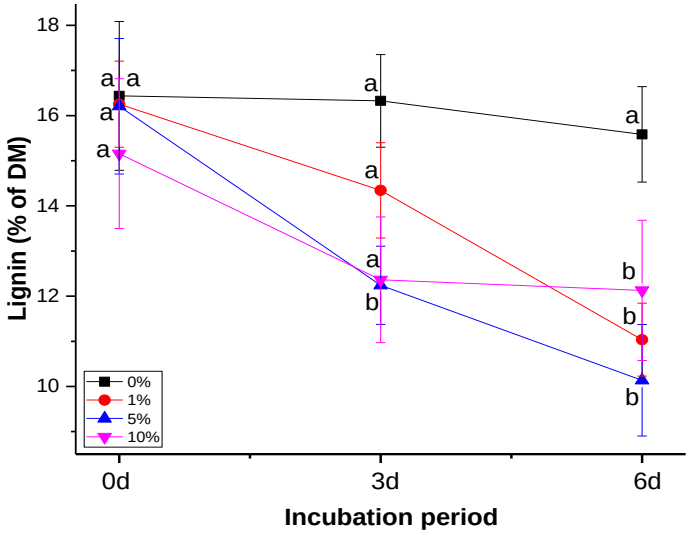

Figure 6. Degradation of lignin fraction in rice straw by *Bacillus subtilis strain* CDB7 isolates.

Lignin

Lignin is a cell wall component in plant and is considered an anti-nutritive component of forages as it cannot be readily fermented by rumen microbes. In terms of energy yield from biomass, the role of lignin depends on the conversion process. It reduces the total digestibility of forage. Depending on the kind of plant, lignin makes up approximately 10 to 25% of lignocellulosic biomass and is the second most abundant natural polymer (Min et al., 2013). The lignin content in the control and treated group of rice straw for 0, 3 and 6 days of incubation is as shown in Figure 6.

The lignin value decreased from 16.3±1.9 to 11.1 ± 0.8, 16.2±1 to 10.1 ± 1.2 and 15.2 ±1.1 to 12.1 ±1.6 in 1, 5, and 10% of inoculums group, respectively. The highest reduction (37.6%) of lignin was found in the 5% level of inoculum group after 6 days of fermentation.

It showed the similarities with Syamsu et al. (2013), who found that the lignin value decreased from 8.13 to 4.96 when treated with microbial starter. Bansi et al. (2012) reported that the enzyme activity of cellulolytic microbes in probiotics caused degradation, reorganization, expansion and break of bonded lignin with the cell wall of rice straw.

Zainudin et al. (2013) found 27 cellulolytic bacterial strains of which 23 strains were closely related to *B. subtilis*, *Bacillus firmus*, *Thermomonospora* species, *Thermobifida fusca*, *Cellulomonas* species, *Ureibacillus thermosphaericus*, *Paenibacillus barengoltzii*, etc. These organisms were known as lignocellulose degrading bacteria and commonly involved in lignocellulose degradation.

Wang et al. (2008) showed that *B. subtilis* has the ability to decompose lignin, phenolic and non-phenolic lignin compounds having low molecular weight. The degradation rate was 9.47% in lignin and 38.8 and 41.84% degradation found in case of cellulose and hemicellulose, respectively.

The results indicated that the structure of rice straw was destroyed when treated with *B. subtilis* and degradation reaction was different in different groups. Several studies (Syamsu et al., 2013; Yoo et al., 2020) have interpreted that this fermentation synergistically and positively improved fermented material quality, which resulted in more soluble carbohydrates, and further improves feed efficiency.

The NDF, ADF and lignin are the least digestible fractions which represent the cellulose, hemicellulose and some ash content of rice straw. The quantity of these fractions reduced significantly when treated with *B. subtilis* strain CDB7 suggesting the availability of more nutrients for ruminants. Thus, this microbial treatment is expected to improve the quality of rice straw which in turn will help ruminants for better performances.

Conclusion

The present study was conducted to isolate the aerobic cellulolytic bacteria from the forest soils of Gazipur, Bangladesh. Out of 10 cellulolytic isolates only one showed the highest hydrolytic capacity and was identified as *B. subtilis* strain CDB7. The hydrolytic capacity suggests the ability of the isolate to produce enzymes that can utilize cellulose and hemicellulose as substrate when available in the media. The solid state fermentation of rice straw with the target isolate showed the significant reduction in ADF, NDF and lignin content which suggests more available sugars in treated rice straw. The cellulolytic bacteria obtained from this study not only degrade the cellulose but also lignin suggesting the diverse role of the isolate. Forest soil could therefore be a good source microbe for degradation of lignocellulosic materials degrading microbes and enzymes. The treatment of rice straw using such cellulolytic bacteria could be adopted by farmers to mitigate the scarcity of ruminant nutrition.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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